Effect of Licorice Versus Tooth Brushing and Dental Flossing on Plaque Accumulation in High Caries Risk Participants: A Randomized Controlled Trial

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Abstract

Objective The purpose of this study was to assess the effect of licorice mouthwash on plaque accumulation in high caries risk individuals.

Materials and methods: A total of 81 participants categorized as high-caries-risk were selected and randomly allocated into three groups: G1 (brushing and flossing only), G2 (Chlorhexidine-Fluoride mouthwash), and G3 (licorice aqueous mouthwash). Plaque index scores were calculated and recorded for each participant at baseline and after one year to evaluate the effect of each preventive measure. Statistical analysis was done using the Kruskal-Wallis test to calculate significance between groups, and the Wilcoxon signed-rank test was used to identify the changes in PI scores after one year within each group.

Result: After one year, there was a statistically significant difference between PI scores in the three groups (P-value <0.001, Effect size = 0.244). Pair-wise comparisons between the groups revealed that G1 (brushing and flossing only) showed the statistically significantly highest score, followed by G3 (licorice aqueous mouthwash plus brushing and flossing), and then G2 (Chlorhexidine-Fluoride mouthwash plus brushing and flossing), which showed the statistically significantly lowest median PI score.

Conclusion: Licorice aqueous extract mouthwash is a natural extract that can improve the plaque score after 1 year follow-up.

KEY WORDS: Dental Caries, Dental Plaque, Glycyrrhiza glabra, Licorice, Liquorice

Introduction

Dental caries is one of the most widely spread diseases all over the world. According to the world health organization, 80% of the population either have the disease or have been suffering from it [1]. Dental caries is considered a dynamic process that occurs due to an improper balance between continuous remineralization and demineralization in the oral cavity. Thus, it is considered a multifactorial disease that requires a tailor-made treatment plan for each patient to stop the effect or remove any of the causative factors.

Nowadays, after understanding the nature of the disease and discovering its various causative factors, it is obvious that its prevention is more beneficial than its management at the next stages, in which surgical intervention might be necessary. Multiple oral rinses are now used to deliver therapeutic constituents to all tooth surfaces to control the disease. However, many side effects have appeared as a result of using different mouthwashes, such as intolerable taste, risk of caries due to fermentation, alcohol content, and discoloration of teeth. The "gold standard" of oral therapeutics is chlorhexidine due to its prolonged broad- spectrum antimicrobial effect, yet it is known to cause altered taste perception, metallic taste, and staining of teeth [2]. However, the various disadvantages of the available oral therapeutics have led to a continuous search for an effective agent with minimal side effects.

There is a great shift towards the use of natural products, which are considered to have many potential bioactive compounds. The use of herbs and plants in curing certain diseases dates back to 1860 AD [3]. Glycyrrhiza glabra Linn, commonly known as licorice (mulethi), is one of the natural products that have been used many years ago by Egyptians, Greeks &

Romans [4]. The Food and Drug Administration (FDA) lists licorice as GRAS (generally regarded as safe) when used as food flavoring and sweetening agent which has an antimicrobial, anti-inflammatory and antiviral activity [5].

Recently, Glycyrrhizin which is the main component of licorice has been proved to combat dental caries by inhibiting glucosyltransferase activity of S. mutans, which is necessary for insoluble glucans formation and biofilm build up [2]. Also, it was found that it reduces enamel dissolution by inhibiting acid production by dental plaque. Moreover, Glycyrrhizin is believed to increase fluoride uptake and reduce enamel demineralization [6]. Thus, it could be highly beneficial and have a great economic value to use it for high caries risk patients. Therefore, the aim of the current study is to evaluate the efficacy of licorice mouthwash in decreasing plaque accumulation compared to the use of chlorhexidine as an adjunctive therapeutic agent to be used with routine teeth brushing and flossing.

Material and methods

1. Study Setting, Ethics Approval and Design

The current study's protocol was reviewed and approved by the Cairo University Faculty of Dentistry's Research Ethics Committee (REC) in October 2018 (Approval Number: 18941) and was registered on ClinicalTrials.gov with a registry's identification number NCT03763578. This study was designed as a parallel group with a ratio of 1:1:1.

2. Recruitment

An announcement was released on the University campus and on the students' study groups on social media to notify them of the research conducted to assess various caries prevention strategies for high-caries-risk patients. Interested participants were invited to be enrolled and the first 81 eligible students were registered in the study after signing an informed consent. Caries risk assessment was conducted on participants using the ADA Chart to determine their susceptibility to caries, as only high-risk individuals were eligible to participate.

3. Patients Preparation

Baseline plaque index was recorded followed by entire supra and sub gingival scaling in addition to polishing of of all teeth to remove the surface biofilm. A comprehensive dental examination was performed visually and all carious teeth were

prepared and restored and any defective restorations were replaced. The purpose of this procedure was to completely eradicate all bacterial niches that could impede proper evaluation of the efficacy of the tested preventive protocols. Participants awareness and training were then practiced following completion of therapy and restoration of all carious lesions. They were instructed how to properly brush and floss their teeth in conjunction with setting a healthier dietary plan.

4. **Randomization**

The 81 participants were then randomly divided and allocated into 3 groups (n=27) by choosing a folded paper containing the number of their assigned group.

5. **Grouping**

5.1. Group 1 (Negative Control: Brushing and Flossing)

Each participant in this group received a set that included a manual soft bristle toothbrush (Oral B, Procter and Gamble, Cincinnati, USA), fluoridated toothpaste "1450 Ppm Fluoride" (Signal, Unilever, UK), and unwaxed dental floss (Oral B, Procter and Gamble, Cincinnati, USA). This set was renewed every three months at the recall visit until the study's completion. They were instructed to adhere to a caries prevention programme for one year, which included brushing their teeth twice a day, after breakfast and before bedtime, for two minutes each time, with a pea-sized fluoridated toothpaste, and manually removing interdental plaque once a day before night brushing with dental floss [7].

5.2. Group 2 (Positive Control: Chlorhexidine-Fluoride Mouthwash)

Each participant in this group received a kit similar to that of the control group in addition to mouthwash bottles containing chlorhexidine (CHX)-fluoride (Emofluor 250 ml, Value pharmacuetical, Cairo, Egypt). They were instructed to adhere to a caries prevention program and were also encouraged to rinse with 15 ml of CHX-Fluoride measured and dispensed twice daily after brushing, through 5 ml plastic syringe for five days monthly for one year.

5.3. Group 3 (Intervention Group: Licorice Mouthwash):

Plaque score	**PI**

Each participant in this group received a similar package to that of the control group and a pre-weighed 75 mg licorice powder packs (Syrian Licorice Powder from Local Market Ragab El Attar, Cairo, Egypt), to be brewed in a total of 150 ml of water and further used as a mouthwash solution on a daily basis for one year. They were also instructed to adhere to a caries prevention program and were instructed to rinse with 15 ml of licorice aqueous extract mouthwash (Twice daily after brushing) by holding it in their mouth for one minute before spitting it out. They were also instructed not to consume any food or drink for 30 minutes following the use of mouthwash.

6. Follow up period

All participants were scheduled to follow up every 3 months for one year from their initial visit to ensure their adherence to the prevention program, monitor any incidence of new carious lesions to be treated, and provide them with their new kits until their subsequent visit.

7. Outcome measured

Plaque score recording

Plaque Index was used to compute and record the plaque scores of participants (PI) **Table 1**. It determines the amount of plaque on the tooth's cervical region. Each tooth had four surfaces recorded: buccal, lingual, and proximal. Six teeth (16, 12, 24, 36, 32, and 44) from each participant were used to calculate the plaque index score. A disclosing solution was used to help the patient visualize the plaque, and it also simplified the process of recording the score. Each participant's index is calculated using six teeth, and each tooth has four records corresponding to the four surfaces buccal, lingual, mesial, and distal. Thus, for each participant, the index was calculated as follows: B+L+M+D= (2+2+2+1) / 4 = 1.75. If the maxillary right first molar (16) scored 1.2, the maxillary right lateral incisor (12) scored 1.6, the maxillary left first bicuspid (24) scored 1.5, the mandibular left first molar (36) scored 1, the mandibular left lateral incisor (32) scored 1.3, and the mandibular right first bicuspid (44) scored 1.2. The patient's index is equal to (1.2 + 1.6 + 1.5 + 1 + 1.3 + 1.2) / 6 = 1.3). Plaque index was recorded for patients before any treatment (baseline data) then was rerecorded after one-year follow-up.

0	Plaque Index, PI < 0.4
1	PI = 0.4-1.0
2	PI = 1.1- 2.0
3	PI > 2.0

Table 1: Plaque Score Index

8. Statistical Analysis

Data were statistically described in terms of mean ± standard deviation (± SD), median and range, or frequencies (number of cases) and percentages when appropriate. Comparison of numerical variables between the study groups was done using Kruskal Wallis test with post-hoc multiple 2-group comparisons. Comparison between baseline and 1y values within the group was done using Wilcoxon signed rank test for paired (matched) samples. Two-sided *p* values less than 0.05 were considered statistically significant. All statistical calculations were done using computer program IBM SPSS (Statistical Package for the Social Science; IBM Corp, Armonk, NY, USA) release 22 for Microsoft Windows.

Results

Plaque scores were recorded for eighty-one participants at baseline however, five patients in brushing and flossing group, four patients of CHX-Fluoride group and three patients of licorice group were lost for one-year follow-up. At baseline; there was no statistically significant difference between PI scores in the three groups (*P*-value = 0.856, Effect size = 0.026). However, after one year, there was a statistically significant difference between PI scores in the three groups (*P*-value <0.001, Effect size = 0.244). Pair-wise comparisons between the groups revealed that the brushing and flossing group showed the statistically significantly highest score. The licorice group showed statistically significantly lower median PI score. The CHX-Fluoride group showed the statistically significantly lowest median PI score as shown in **Table 2.**

Time	PI scores	Standard preventive measures (n = 22)	CHX-Fluoride (n = 23)	Licorice (n = 24)	*P*-value	Effect size *(Eta squared)*
Base line	Median (Range)	3 (1-3)	3 (0-3)	3 (0-3)	0.856	0.026
	Mean (SD)	2.59 (0.67)	2.43 (0.84)	2.42 (0.97)		
1 year	Median (Range)	3 (1-3) ^{A}	1 (0-2) ^{C}	2 (0-3) ^{B}	<0.001*	0.244
	Mean (SD)	2.5 (0.67)	1.26 (0.75)	1.79 (1.18)		

Table 2: Descriptive Statistics and Results of Kruskal-Wallis Test for Comparison Between PI Scores in the Three Groups, *: Significant at $P \leq 0.05$.

As regards standard preventive measures group; there was no statistically significant change in PI scores after one year (*P*-value = 0.317, Effect size = 0.213) while, CHX-Fluoride as well as Licorice groups; there was a statistically significant decrease in PI scores after one year (*P*-value <0.001, Effect size = 0.843) and (*P*-value = 0.016, Effect size = 0.512), respectively as in **Table 3**.

Time	PI scores	Standard preventive measures (n = 22)	CHX-Fluoride (n = 23)	Licorice (n = 24)
Base line	Median (Range)	3 (1-3)	3 (0-3)	3 (0-3)
	Mean (SD)	2.59 (0.67)	2.43 (0.84)	2.42 (0.97)
1 year	Median (Range)	3 (1-3)	1 (0-2)	2 (0-3)
	Mean (SD)	2.5 (0.67)	1.26 (0.75)	1.79 (1.18)
P-value		0.317	<0.001*	0.016*
Effect size *(d)*		0.213	0.843	0.512

Table 3: Descriptive Statistics and Results of Wilcoxon Signed-Rank Test for the Changes in PI Scores After One Year Within Each Group**,** *: Significant at $P \leq 0.05$

Discussion

The aim of this study is to assess various plaque control approaches, as prevention is crucial for keeping healthy tooth surfaces from developing new caries lesions, arresting the progression of already active lesions, and halting the progression of dormant lesions. Additionally, preventive measures are taken to reduce patients' risk status. Effective preventive programs improve patient satisfaction, involvement, and well-being and are favored by patients due to their minimal invasive nature. As Banerjee et al. point out that standard surgical tooth removal and dental restoration address only the symptoms of advanced caries disease and not the illness's basic causes [8]. This also matches with Urquhart et al. systematic review, which stated that the primary goal of a caries management plan should be to prevent caries from developing [9].

The study population consisted of high-caries-risk young Egyptians aged 18-25, as Egyptians were categorized as a high-caries-risk group by the World Health Organization in 2015 [10]. The purpose of this study was to determine the efficiency of aqueous licorice extract in the form of mouthwash in preventing dental plaque accumulation and thus preventing dental caries. The novel notion of employing natural products to prevent dental caries. Aqueous extracts of the licorice were used in this study instead of ethanolic extract as this study meant at examining the antimicrobial activity of the extract in the crude forms. Licorice mouthwash was compared to mouthwash containing chlorhexidine-fluoride as a positive control as combining both chlorhexidine-fluoride was proven by Sajadi et al. [11] and Dehghani et al. [12] to be effective in caries prevention rather than chlorhexidine alone.

In our clinical trial, a very low concentration of 0.5mg/ml (50% of aqueous licorice extract) was used once daily, as a mouthwash only, without swallowing or ingestion. According to the World Health Organization recommendations, a maximum daily dose of 100 mg/kg of licorice containing glycyrrhizin is considered safe. Higher concentrations than the aforementioned 50% licorice might not be palatable or accepted by patients due to its consistency specially that the used aqueous extract was not sieved to avoid the loss of any of its components or active ingredients.

The control group got normal preventive treatments, which included brushing twice daily after breakfast and at bedtime with fluoridated toothpaste and once daily interdental cleaning at bedtime. Brushing with fluoride toothpaste is unquestionably the most prevalent method of plaque control in use today which is consistent with the American Dental Association home care recommendation form, that recommends using fluoride toothpaste to prevent caries and interdental cleaning to remove biofilm mechanically.

Our results showed that in the brushing and flossing group; there was no statistically significant change in PI scores after one year. As regards CHX-Fluoride as well as licorice groups; there was a statistically significant decrease in PI scores after one year. This could be justified by saliva-coating which strongly decreases S. mutans adhesion to polystyrene surfaces, thus weakening bacterial adhesion to the substratum by lowering the surface free energy. Additionally, Ahn et al. [13] discovered that it may be related to altered bacterial cell surface features and resulted in a small increase in the aggregation of S. mutans UA159. Thus, licorice limit biofilm formation by enhancing the cell surface hydrophobicity and aggregation. So, the reduced production of biofilms can be attributed to both growth inhibition and decreased bacterial adherence.Our findings concur other research [4,14,15] demonstrating that licorice and specifically its active ingredient glycyrrhizin ceased bacterial growth and triggered plaque formation in the presence of sucrose and at a concentration of 0.5-1 percent, plaque formation was virtually completely suppressed.

Licorice roots have been used traditionally as teeth cleaning twigs and as teething sticks due to their numbing and cooling impact on newborns' gums. It can also be added to newborn swabs and pacifiers to provide a non-cariogenic, sweet-tasting alternative to honey-dipped pacifiers, according to Jain et al. [2] the form of administration of licorice to subjects is a critical factor for achieving a prolonged duration of action to have a relatively sustained effect comparable to that of chlorhexidine. Thus, it might be suggested that licorice would show better results if administered as a hard candy, chewing gum, viscous suspension or an oral gel that would have a prolonged release in the mouth, enhancing its sustained impact greatly rather than easily being washed away when used in the form of a mouthwash.

Moreover, our results showed a greater mean of plaque scores in the licorice group compared to that of CHX-Fluoride group which might also be attributed to the use of an aqueous extract of licorice which was claimed by Ajagannanavar et al. [15] to be comparatively of lower efficiency

than ethanolic extracts, in which alcoholic extracts might have better solubility with an abundance of more active ingredients rather than the use of aqueous forms.

Conclusion

Licorice aqueous extract mouthwash has antibacterial properties that might be effective at higher concentrations and when kept for a longer duration in the oral cavity when used for dental caries prevention. This clinical experiment has significant limitations that should be addressed in future research. The sample of young individuals, the majority of whom are students, could be broadened to include older participants to examine how working life and associated stress affect their oral healthcare habits. Additionally, future research could incorporate diverse geographical representations, as well as longer follow-up periods. The latter complexes provide a fertile research area that requires intense effort to achieve the simplest hygiene form, the highest level of patient compliance, and the most cost-effective routine. Moreover, higher licorice concentrations in different forms rather than a mouthwash can be used in further clinical studies which is mandatory to confirm whether licorice consumption might show microbial resistance of microorganisms over time and observe any possible oral side effects.

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